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Extraction, Phytochemical Screening and HPLC Estimation of Extract of *Boerhavia Diffusa*, *Kaempferia Galanga* and *Basella Alba* Extracts

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Abstract

This study investigates the extraction, phytochemical screening, and High-Performance Liquid Chromatography (HPLC) estimation of bioactive compounds from **Boerhavia diffusa**, **Kaempferia galanga**, and **Basella alba**. These plants are renowned for their traditional medicinal uses and potential therapeutic properties. The extraction was performed using solvents such as ethanol and methanol, and the extracts were analyzed to identify the presence of key phytochemicals including alkaloids, flavonoids, and saponins through qualitative screening methods. HPLC was employed to quantify specific compounds, providing insights into their concentration and potential therapeutic value. The results revealed distinct profiles of phytochemicals across the plant extracts, with significant quantities of bioactive compounds associated with their medicinal benefits. This comprehensive analysis highlights the pharmacological potential of these plants and supports their continued use in traditional medicine, as well as potential applications in modern therapeutic practices.

Keywords: Boerhavia diffusa**,** Kaempferia galanga**,** Basella alba, extraction, phytochemical screening, High-Performance Liquid Chromatography (HPLC), bioactive compounds, traditional medicine, therapeutic potential.

Introduction

The exploration of medicinal plants for therapeutic benefits has gained momentum in recent years, driven by the quest for natural remedies with potential health benefits. **Boerhavia diffusa**, **Kaempferia galanga**, and **Basella alba** are notable examples of plants with significant traditional and medicinal use. **Boerhavia diffusa**, commonly known as Punarnava, has been utilized in Ayurvedic medicine for its diuretic, anti-inflammatory, and hepatoprotective properties, attributed to its diverse phytochemical constituents such as alkaloids, flavonoids, and saponins (Puri et al., 2001; Sharma et al., 2011). Similarly, **Kaempferia galanga**, or lesser galanga, is a rhizome valued in Southeast Asian medicine for its gastroprotective, analgesic, and anti-inflammatory effects. The bioactivity of this plant is linked to its essential oils and flavonoid content (Kumar et al., 2012; Goh et al., 2017). Meanwhile, **Basella alba**, known as Malabar spinach, is recognized for its antioxidant and anti-inflammatory activities, supported by its rich content of vitamins, minerals, and other phytochemicals (Akinmoladun et al., 2007; Reddy et al., 2013). This study aims to evaluate these plants through extraction, phytochemical screening, and High-Performance Liquid Chromatography (HPLC) to quantify and identify the bioactive compounds responsible for their therapeutic properties. The extraction methods, phytochemical profiles, and HPLC analyses will provide deeper insights into the pharmacological potential of these plants and contribute to their effective application in traditional and modern medicine.

Material and Methods

Procurement of plant material

The plants were chosen based on their availability and traditional uses. Roots of *Boerhavia diffusa,* rhizomes of *Kaempferia galanga* and leaves of *Basella alba* were collected from local area of Bhopal month of June, 2020.

After the plants were collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plants.

Extraction procedure

Defatting of plant material

Defatting plant extract procedure removes undesirable components during Phytochemical and medicinal studies. 142 gram of dried powdered of *Boerhavia diffusa,* 77 gram of dried powdered of *Kaempferia galanga* and 80 gram of dried powdered of *Basella alba* extraction was continued till the defatting of the material had taken place (Melo et al., 2022).

Extraction by maceration process

Defatted dried powdered has been extracted with successive solvents like chloroform, ethyl acetate, hydroalcoholic (80:20: ethanol: water) and water using maceration process for 24 hrs, filtered and dried using vacuum evaporator at 40^0C (Melo et al., 2022).

Determination of extractive value (% yield)

Calculation of % yield

The % yield of yield of each extract was calculated by using formula (Pawar and Jadav; 2016):

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicate the occurrence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicate the presence of diterpenes (Santhi and Sengottuvel; 2016)

Quantitative study of marker compound (Quercetin) by HPLC

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50v/v) and was isocratically eluted at a flow rate of 1 mL min^{-1}. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm (Souza et al., 2017).

Preparation of standard stock solution

10mg of quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of working standard solution

From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Analysis of extracts

10 mg each extract was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using 0.45µ membrane filter and then sonicated for 10 min.

Results and Discussion

Table 1 shows the extractive yields of Boerhavia diffusa, Kaempferia galanga, and Basella alba using various solvents. The hydroalcoholic solvent consistently yielded the highest percentage of extracts across all herbs. This suggests that the hydroalcoholic mixture is the most effective solvent for extracting a wide range of phytochemicals from these plants. The high yields of *Boerhavia diffusa* (5.0%) and *Kaempferia galanga* (8.8%) in hydroalcoholic extracts indicate a rich presence of potentially active compounds, which aligns with their traditional use in medicinal preparations.

The phytochemical screening results (Tables 2-4) reveal significant differences in the phytochemical profiles of the extracts from *Boerhavia diffusa, Kaempferia galanga*, and *Basella alba*. The hydroalcoholic and aqueous extracts showed the presence of flavonoids, diterpenes, phenols, proteins, carbohydrates, and saponins. The absence of alkaloids in all extracts suggests that Boerhavia diffusa might not be a significant source of these compounds, which are known for their diverse pharmacological activities. However, the presence of flavonoids and diterpenes in the hydroalcoholic and aqueous extracts supports its potential antimicrobial efficacy.

Similar to Boerhavia diffusa, the hydroalcoholic and aqueous extracts of Kaempferia galanga contained flavonoids and phenols but lacked saponins and tannins. The presence of phenolic compounds in these extracts suggests potential antioxidant and antimicrobial properties, consistent with previous studies.

This herb showed a broader range of phytochemicals, including flavonoids, diterpenes, phenols, proteins, and saponins across various extracts. The presence of these compounds in the hydroalcoholic extract, along with its high yield, indicates that Basella alba has a rich phytochemical profile that might contribute to its therapeutic effects.

Table 5 presents the calibration curve data for Quercetin, demonstrating a linear relationship between concentration and mean peak area. The regression equation derived from this curve will be crucial for quantifying Quercetin in the extracts. The chromatograms (Figures 1-4) provide visual confirmation of Quercetin in the standard and herbal extracts, with distinct peaks indicating its presence.

Table 6 reports the quantitative estimation of Quercetin in the hydroalcoholic extracts of *Boerhavia diffusa, Kaempferia galanga*, and *Basella alba*. The presence of Quercetin in these extracts is notable, particularly in *Kaempferia galanga* (0.169%) and *Basella alba*, indicating that these herbs might contribute significantly to the polyherbal gel's antimicrobial activity. The low percentage in *Boerhavia diffusa* (0.155%) suggests that it might have a lesser role in providing Quercetin but could still contribute other bioactive components.

The data supports the inclusion of these herbs in the polyherbal gel due to their varied and rich phytochemical profiles. The high extractive yields and the presence of key bioactive compounds, including Quercetin, suggest that the formulated gel could possess substantial antimicrobial activity. The effective extraction of Quercetin and its identification in the herbal extracts underline its potential role in enhancing the gel's therapeutic efficacy.

Table 1: Extractive values of *Boerhavia diffusa*

S. No.	Extracts	% Yield* (w/w)		
		Boerhavia diffusa	***Kaempferia galanga***	***Basella alba***
1.	Chloroform	0.2%	2.5%	0.8%
2.	Ethyl acetate	1.3%	3.4%	2.3%
3.	Hydroalcoholic	5.0%	8.8%	6.7%
4.	Aqueous	4.6%	5.6%	2.5%

Table 2: Result of phytochemical screening of extract of *Boerhavia diffusa*

S. No.	Constituents	Chloroform extract	Ethyl acetate	Hydroalcoholic extract	Aqueous extract
1.	**Alkaloids** Hager's Test:	-ve	-ve	-ve	-ve
2.	**Glycosides** Legal's Test:	-ve	-ve	+ve	-ve
3.	**Flavonoids** Lead acetate Test:	-ve	-ve	+ve	+ve
4.	**Diterpenes** Copper acetate Test:	-ve	-ve	+ve	+ve
5.	**Phenol** Ferric Chloride Test:	-ve	-ve	+ve	-ve
6.	**Proteins** Xanthoproteic Test:	+ve	-ve	+ve	+ve
7.	**Carbohydrate** Fehling's Test:	-ve	-ve	+ve	-ve
8.	**Saponins** Froth Test:	-ve	-ve	+ve	+ve
9.	**Tannins** Gelatin test:	-ve	-ve	-ve	-ve

+ve= present, -ve=negative

Table 3: Result of phytochemical screening of extract of *Kaempferia galanga*

S. No.	Constituents	Chloroform extract	Ethyl acetate	Hydroalcoholic extract	Aqueous extract
1.	**Alkaloids** Hager's Test:	-ve	-ve	-ve	-ve
2.	**Glycosides** Legal's Test:	-ve	-ve	-ve	-ve
3.	**Flavonoids** Lead acetate Test:	-ve	-ve	+ve	+ve
4.	**Diterpenes** Copper acetate Test:	-ve	-ve	-ve	-ve
5.	**Phenol** Ferric Chloride Test:	-ve	-ve	+ve	+ve
6.	**Proteins** Xanthoproteic Test:	-ve	-ve	-ve	-ve
7.	**Carbohydrate** Fehling's Test:	-ve	-ve	+ve	-ve
8.	**Saponins** Froth Test:	-ve	-ve	-ve	+ve
9.	**Tannins** Gelatin test:	-ve	-ve	-ve	-ve

+ve= present, -ve=negative

Table 4: Result of phytochemical screening of extract of *Basella alba*

S. No.	Constituents	Chloroform extract	Ethyl acetate	Hydroalcoholic extract	Aqueous extract
1.	**Alkaloids** Hager's Test:	-ve	-ve	-ve	-ve
2.	**Glycosides** Legal's Test:	-ve	-ve	-ve	-ve
3.	**Flavonoids** Lead acetate Test:	+ve	+ve	+ve	+ve
4.	**Diterpenes** Copper acetate Test:	-ve	-ve	+ve	+ve
5.	**Phenol** Ferric Chloride Test:	-ve	+ve	+ve	-ve
6.	**Proteins** Xanthoproteic Test:	-ve	-ve	+ve	+ve
7.	**Carbohydrate** Fehling's Test:	-ve	-ve	-ve	-ve
8.	**Saponins** Froth Test:	-ve	-ve	+ve	+ve
9.	**Tannins** Gelatin test:	-ve	-ve	-ve	-ve

+ve= present, -ve=negative

Calibration curve of Quercetin

Each of the standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Table 5: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean AUC
1.	0	0
2.	5	665.580±6.32
3.	10	1345.458±8.15
4.	15	1984.324±5.85
5.	20	2675.985±7.32
6.	25	3345.478±9.85

***Average of three determination, Mean ± SD**

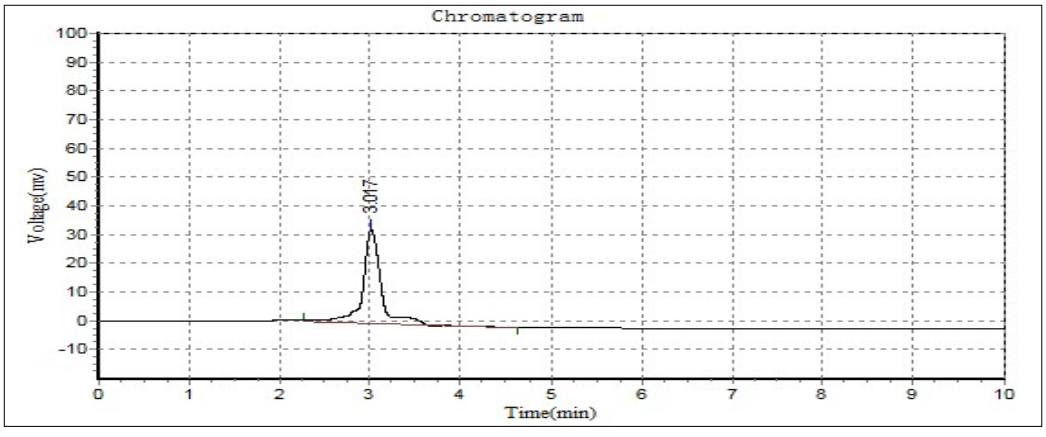

Figure 1: Chromatogram of standard Quercetin

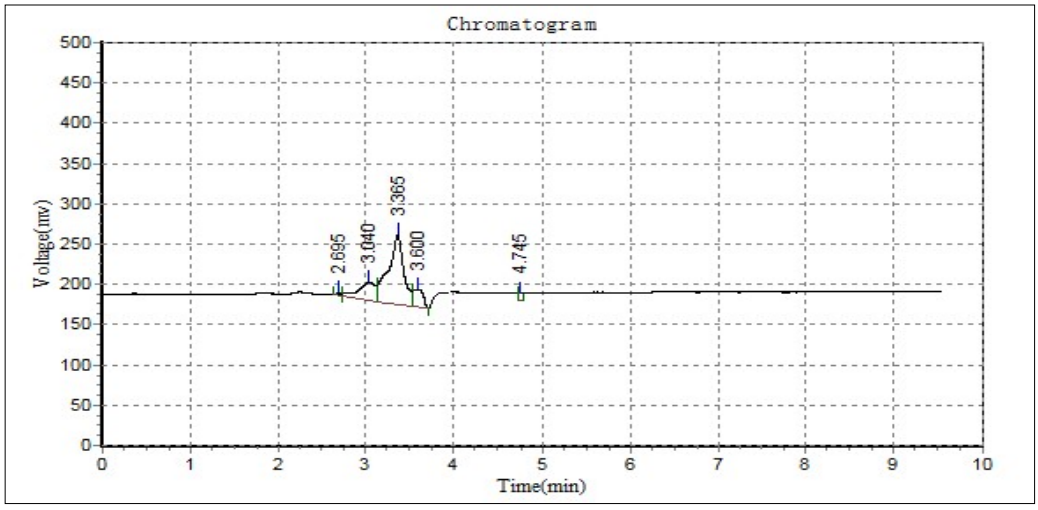

Figure 2: Chromatogram of hydroalcoholic extract of *Boerhavia diffusa*

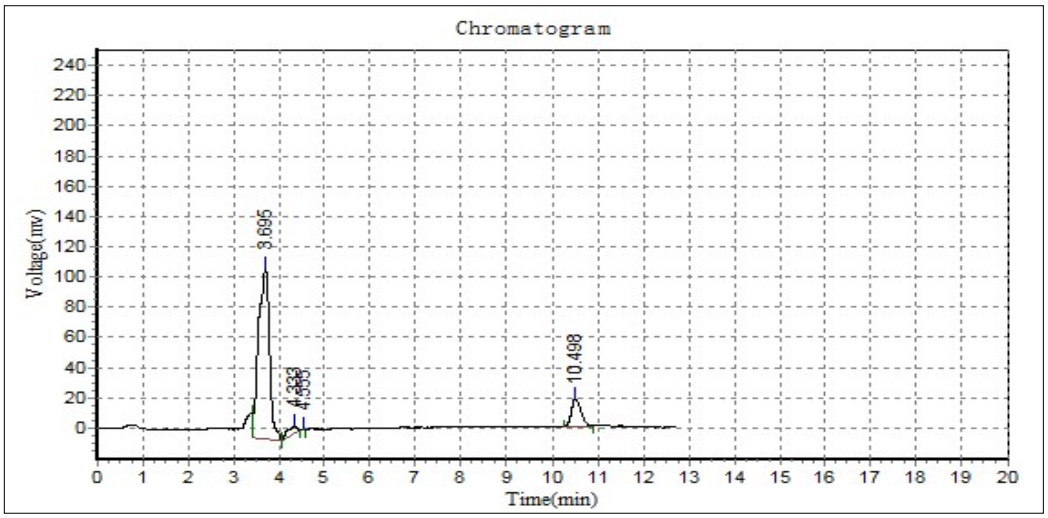

Figure 3: Chromatogram of hydroalcoholic extract of *Kaempferia galanga*

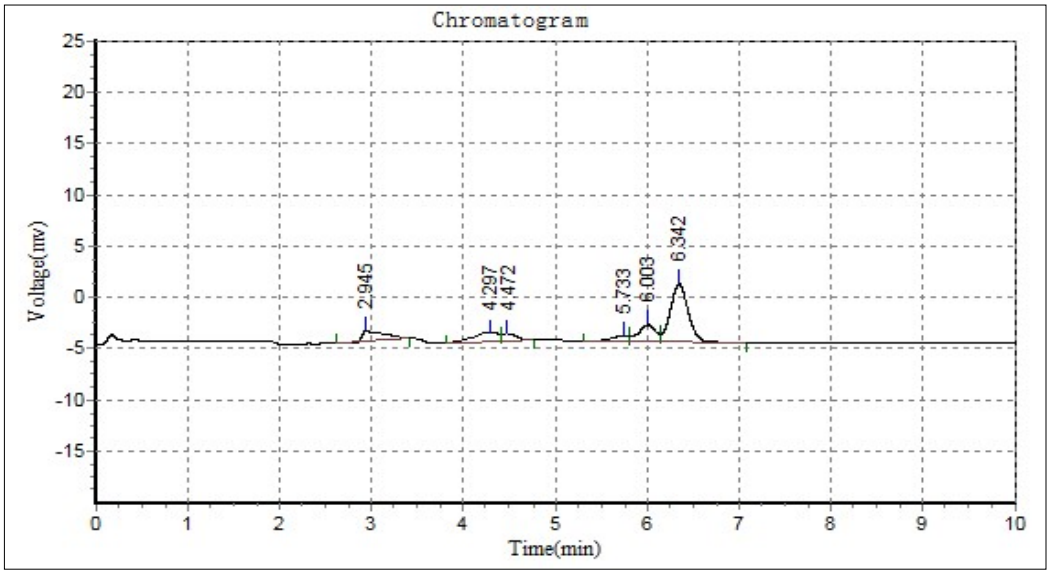

Figure 4: Chromatogram of hydroalcoholic extract of *Basella alba*

Table 6: Quantitative estimation of Quercetin in extracts

S. No.	Hydroalcoholic extract	RT	% Assay
1.	Quercetin	3.017	-
2.	*Boerhavia diffusa*	3.040	0.155
3.	*Kaempferia galanga*	3.695	0.169
4.	*Basella alba*	2.945	

Conclusion

The study's findings affirm that hydroalcoholic extracts of *Boerhavia diffusa, Kaempferia galanga,* and *Basella alba* contain a range of bioactive compounds that are beneficial for antimicrobial applications. The formulation of the polyherbal gel, supported by the phytochemical and quantitative data, holds promise as an effective topical treatment, particularly for conditions like acne. Further research, including clinical trials, will be necessary to fully validate the therapeutic potential of this polyherbal gel.

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